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Abstract

Background: The aim of this randomised, three-period, three-treatment crossover trial was to examine the acute effects of regularly breaking up seated office work with short bouts of standing or light-intensity walking on postprandial interstitial glucose concentration.

Methods: Seventeen middle-aged office workers performed three five-hour trial conditions at their workplace in a random order: 1) uninterrupted sitting; 2) sitting interrupted by two minutes of standing every 20 minutes; and 3) sitting interrupted by two minutes of light-intensity walking every 20 minutes. Participants consumed two standardised test drinks at the start of each trial condition and an iPro2 continuous glucose monitoring system (CGMS) recorded average interstitial glucose concentration every five minutes for the duration of the study.

Results: Five-hour interstitial glucose incremental area under the curve (iAUC) was 55.5% lower after sitting interrupted by light-intensity walking compared with after uninterrupted sitting (95% CI, -104.2% to -6.8%). There was also a suggestion of a beneficial effect of regular standing breaks, particularly in overweight men, although they were not as effective as the walking breaks (mean difference [95% CI], -29.6% [-73.9% to 14.7%]).

Conclusions: Regularly breaking up prolonged sitting lowers postprandial glycemia in middle-aged adults without metabolic impairment.

Background

A number of recent experimental studies have shown that regularly breaking up prolonged sitting with short bouts of light- or moderate-intensity walking lowers postprandial glycemia compared with uninterrupted sitting.¹⁻⁵ In fact, despite equal amounts of total physical activity, Peddie et al. (2013)⁴ found that regular activity breaks were more effective than a continuous bout of activity at lowering postprandial glycemia in 70 healthy, young adults.

However, standing breaks may also be beneficial because, unlike sitting, standing involves contraction of the postural skeletal muscles. Tikkanen et al. (2013)⁶ used electromyography (EMG) to show that thigh muscle activity was more than doubled during standing compared with during sitting. Furthermore, self-reported total daily standing time has been shown to be inversely associated with the risk of all-cause and cardiovascular disease (CVD) mortality in a large, nationally representative sample of Canadian adults, even after adjusting for other risk factors, including age, sex, smoking status and moderate-to-vigorous-intensity physical activity (MVPA).⁷

To our knowledge, six experimental studies to date have examined the acute effects of standing on postprandial glycemia. Three examined prolonged bouts of standing (30 minutes⁸, 45 minutes⁹ and four hours¹⁰, respectively) and three examined short bouts (two minutes^{1,5} and five minutes³, respectively). However, their results are conflicting, with three reporting a beneficial effect of standing^{3,8,10} and three reporting no effect.^{1,5,9} Therefore, the primary aim of the current study was to examine the acute effects of regularly breaking up seated office work with short bouts of standing or light-intensity walking on postprandial interstitial glucose concentration. The secondary aim was to investigate the acceptability of two interventions that break up prolonged sitting in the workplace.

Methods

Study overview

This randomised, three-period, three-treatment crossover trial was approved by the University of Bristol Faculty of Science Human Research Ethics Committee and is reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Supplementary Material 1). All participants provided written informed consent.

The researcher visited each participant at their workplace on three separate days over a period of a week (Monday, Wednesday and Friday) and participants performed three five-hour trial conditions in a random order: 1) uninterrupted sitting; 2) sitting interrupted by standing; and 3) sitting interrupted by light-intensity walking. There was a 24-hour wash-out period between each trial condition to minimise potential carryover effects.

Participants and enrolment process

University of Bristol employees were recruited between June 2014 and February 2015 by direct advertising (Figure 1). Eligibility criteria included aged 45 to 65 years and employed full-time in an entirely sedentary or semi-sedentary occupation. An entirely sedentary occupation was defined as chair-bound most of the day, whilst a semi-sedentary occupation was defined as intermittently standing and chair-bound, but without substantial walking or physical labour.¹¹ Exclusion criteria were non-English speaking, pregnancy, clinically diagnosed diabetes, taking lipid-lowering medication and major illness or injury (acute or chronic).

Study protocol

Figure S1 shows the study protocol. Participants were instructed to refrain from any MVPA, alcohol and caffeine for 24 hours before each trial condition. Furthermore, at approximately 7pm on the evening before each trial condition, participants consumed a

standardised meal at home (chilli con carne with rice [637kcal; 44% of energy from carbohydrate, 33% from fat and 18% from protein] or penne with tomato [610kcal; 50% of energy from carbohydrate, 36% from fat and 12% from protein]). After finishing the meal, participants performed an overnight fast.

Participants were requested not to walk or cycle to work on the morning of each trial condition. The researcher arrived at each participant's workplace at approximately 9.45am and participants consumed two standardised 200-mL test drinks within 15 minutes (Nutricia Fortisip, Nutricia Ltd., Trowbridge, Wiltshire, UK). In total, the two test drinks contained 600kcal of energy, 73.6g of carbohydrate, 23.6g of protein and 23.2g of fat. These particular test drinks were chosen for two main reasons: 1) to simulate a mixed meal and 2) because fat slows down gastrointestinal emptying, spreading the postprandial glucose responses over more of the five-hour monitoring period.¹² After finishing both of the test drinks, participants performed one of the three five-hour trial conditions. Participants performed one trial condition on Monday and the remaining two on Wednesday and Friday, respectively. The researcher directly supervised the participants throughout all three trial conditions to make sure they were complying with the protocols. The three trial conditions were as follows:

1. **Uninterrupted sitting:** participants performed five hours of uninterrupted seated office work, only rising from their chair to use the toilet.
2. **Sitting interrupted by standing:** participants rose from their chair every 20 minutes and stood as still as possible at their desk for two minutes. The researcher was responsible for timekeeping and told participants when to stand up and sit down. Participants interrupted their seated office work on 14 occasions, resulting in a total of 28 minutes of standing.
3. **Sitting interrupted by light-intensity walking:** participants rose from their chair every 20 minutes and walked up and down a nearby corridor at a self-perceived light intensity for two minutes (Borg rate of perceived exertion [RPE] rating of 9). Participants were

instructed to avoid climbing up any stairs as this may have increased thigh muscle activity, and hence energy expenditure, more than intended.⁶ The researcher was responsible for timekeeping and told participants when to start and stop walking. Participants interrupted their seated office work on 14 occasions, resulting in a total of 28 minutes of walking.

Activity monitoring

Participants wore two activity monitors simultaneously from the start of trial condition one until the end of trial condition three: an activPAL 3c physical activity monitor (PAL Technologies Ltd., Glasgow, UK) and an ActiGraph GT3X+ accelerometer (ActiGraph LLC, Pensacola, Florida, USA). The activPAL was attached directly to the skin on the midline of the anterior aspect of the thigh using a double-sided, hypoallergenic, hydrogel adhesive pad (i.e. a PALstickie), whilst the ActiGraph was worn on an elastic belt around the waist and positioned on the hip. Participants were instructed to wear both activity monitors during waking hours, except during water-based activities.

The activPAL 15-second epoch data was used to confirm compliance with the trial condition protocols (Table S1). The ActiGraph 15-second epoch data was used to examine the total amount of time spent sedentary (<100 counts per minute [cpm]), in light-intensity physical activity (LPA; 100 to 1,951 cpm) and in MVPA ($\geq 1,952$ cpm) during each of the 24-hour wash-out periods (Tuesday and Thursday). At least 600 minutes of accelerometer wear time were required to be included in the final analyses. Non-wear time was defined as at least 60 consecutive minutes of zero activity counts, with allowance for up to two minutes of non-zero activity counts.

Continuous glucose monitoring

From the start of trial condition one until the end of trial condition three, average interstitial glucose concentration was recorded every five minutes using an iPro2 continuous glucose monitoring system (CGMS; Medtronic MiniMed, Inc., Northridge, California, US). One hour prior to consumption of the test drinks on the morning of trial condition one, each participant had an Enlite glucose sensor inserted into the subcutaneous tissue of their abdomen by the researcher. An iPro2 digital recorder (iPro2) was then connected to the glucose sensor and stuck to the skin of the abdomen by an adhesive tab. The glucose sensor continuously measured glucose concentration in the interstitial fluid, whilst the iPro2 recorded average interstitial glucose concentration every five minutes.

After removal of the glucose sensor at the end of trial condition three, glucose data were uploaded from the iPro2 to the manufacturer's software (CareLink iPro Therapy Management Software for Diabetes). From there, capillary glucose values obtained using the finger prick method were used to retrospectively calibrate the glucose values obtained from the CGMS. Participants were instructed to perform four finger prick tests per day, before breakfast, lunch, dinner and bed, using an Accu-Chek Aviva blood glucose meter (Roche Diagnostics Ltd., Burgess Hill, West Sussex, UK). Finally, calibrated glucose data were exported into Microsoft Excel for analysis.

Acceptability questionnaire

At the end of trial condition three, participants completed a questionnaire about the acceptability of the two interventions that broke up prolonged sitting in the workplace, including a question about the most acceptable intervention.

Randomisation

The random order for the three trial conditions was computer-generated by the researcher using pseudorandom numbers. There were six possible sequences (Figure 1). Participants were told their trial condition order at the preliminary assessments visit.

Sample size calculation

The sample size calculation was performed using PASS 14 software. Based on a difference in means of 24% between uninterrupted sitting and the two interrupted sitting trial conditions, respectively,² we estimated that a sample of 17 participants, each measured at three time points, was required to achieve 80% power to detect differences between the means at a 0.05 significance level. The between-subject standard deviation at each time point was assumed to be 48%² and the correlation structure of the covariance matrix was to have all correlations equal at 0.75.

Statistical analysis

The primary outcome in this study is five-hour interstitial glucose incremental area under the curve (iAUC). IAUC includes the area above baseline only (i.e. the area above fasting glucose concentration). Any area below baseline is ignored, rather than subtracted, thus eliminating the possibility of negative areas.¹³ IAUC was calculated in Microsoft Excel using the trapezium rule¹⁴ and a difference of 20% was deemed to be clinically meaningful in line with a previous study.¹⁵ Sensitivity analyses were conducted to investigate whether subtracting the area below baseline (i.e. positive iAUC) or including the area below baseline (i.e. total AUC) significantly affected the results of the study.

Generalised estimating equation (GEE) models were used to examine the differential effects of the three trial conditions on the outcomes. Each model had an exchangeable within-group correlation structure to account for dependency in the data (i.e. repeated measures) and

the quasiliikelihood under the independence model criterion (QIC) method¹⁶ was used to select the best subset of the following covariates: order (six levels), previous trial condition (four levels, including no trial condition) and period (three levels). Post-estimation pairwise comparisons, with adjustment for multiple comparisons (Sidak method), were used to examine the differential effects of pairs of trial conditions on the outcomes. All statistical analyses were performed using Stata 14 software.

Results

Trial CONSORT diagram

The trial CONSORT diagram is shown in Figure 1. Forty-four people were assessed for eligibility, but only 17 took part in the study. All 17 participants completed all three trial conditions. However, only 13 had complete glucose data. Two participants had missing data during uninterrupted sitting, one during sitting interrupted by light-intensity walking and one during both uninterrupted sitting and sitting interrupted by standing. An intention to treat analysis was undertaken to minimise bias. Therefore, all 17 participants were included in the final analyses. Participant characteristics are reported in Table S2. Men had a higher body mass index (BMI) and larger waist circumference (WC) compared with women, but there were no differences in the remaining characteristics.

Participants spent less time sedentary and more time in LPA on the day before sitting interrupted by standing compared with before the remaining two trial conditions. However, there were no differences in the total amount of time spent in MVPA (Table S3). Fasting interstitial glucose concentration was higher before sitting interrupted by light-intensity walking compared with before uninterrupted sitting (mean difference [95% CI], 0.4 mmol/L [-0.05 to 0.9 mmol/L]; $p = 0.090$). However, there were no differences between the remaining trial conditions (Table S4).

Glucose response curves

Figure 2 shows the average glucose response to the test drinks during each of the five-hour trial conditions. Interstitial glucose concentration was lower throughout the entire five-hour monitoring period during the two interrupted sitting trial conditions compared with during uninterrupted sitting. Apart from the first 25 minutes and the last 55 minutes, interstitial glucose concentration was also lower during sitting interrupted by light-intensity walking compared with during sitting interrupted by standing.

Glucose iAUC

After adjustment for order, previous trial condition and period, five-hour interstitial glucose iAUC was 55.5% lower after sitting interrupted by light-intensity walking compared with after uninterrupted sitting (mean difference [95% CI], $-119.6 \text{ mmol}\cdot\text{L}^{-1}\cdot 5 \text{ hrs}^{-1}$ [-224.6 to -14.6]; $p = 0.020$; Figure 3A). There was also a suggestion that glucose iAUC was lower after sitting interrupted by standing compared with after uninterrupted sitting (mean difference [95% CI], $-63.8 \text{ mmol}\cdot\text{L}^{-1}\cdot 5 \text{ hrs}^{-1}$ [-159.2 to 31.7]; $p = 0.297$), as well as after sitting interrupted by light-intensity walking compared with after sitting interrupted by standing (mean difference [95% CI], $-55.8 \text{ mmol}\cdot\text{L}^{-1}\cdot 5 \text{ hrs}^{-1}$ [-154.0 to 42.4]; $p = 0.438$). A similar pattern of results was observed when the first two hours after the test drinks (postprandial glucose iAUC; Figure 3B) and the remaining three hours (preprandial glucose iAUC; Figure 3C) were analysed separately, although the statistical evidence was stronger for the postprandial phase.

Glucose positive iAUC

Subtracting the area below baseline, rather than ignoring it, did not significantly affect the results of the study (Figure S2).

Glucose total AUC

There were no differences in interstitial glucose total AUC between the three trial conditions (Figure S3).

Stratified analyses

The effects of the two interventions on five-hour interstitial glucose iAUC did not significantly differ by occupation or self-reported physical activity level (p for interaction = 0.870 and 0.578, respectively). However, there was a suggestion that both interventions were more beneficial in men than in women (p for interaction = 0.295) and in overweight participants than in normal-weight participants (p for interaction = 0.104; Table S5 and Figure S4).

Acceptability of the interventions

The participants' responses to the acceptability questionnaire and supporting quotes are reported in Table S6 and Table S7, respectively. The majority of participants (eight or 47.1%) thought that sitting interrupted by standing was the most acceptable intervention, primarily because it was less disruptive to their work. Only 11.8% reported a reduction in productivity compared with before the study, in contrast to 52.9% during sitting interrupted by light-intensity walking. Four participants (23.5%) thought that sitting interrupted by light-intensity walking was the most acceptable intervention, mainly because it involved a change of scenery and gave them the opportunity to stretch their legs, whilst the remaining five (29.4%) thought that both interventions were equally acceptable. The majority of participants thought that the duration of the breaks was acceptable (88.2% and 94.1% during sitting interrupted by light-intensity walking and sitting interrupted by standing, respectively). The frequency of the breaks was also found to be acceptable during sitting interrupted by standing (64.7%), but too frequent during sitting interrupted by light-intensity walking (82.4%).

Discussion

The main finding from the current study was that breaking up seated office work every 20 minutes with two minutes of light-intensity walking lowered postprandial interstitial glucose concentration by clinically meaningful amounts compared with five hours of uninterrupted seated office work in 17 middle-aged office workers. There was also a suggestion of a beneficial effect of regular standing breaks, particularly amongst the male participants who were more overweight than the females, although they were not as effective as the walking breaks.

Similar increases in postprandial glycemia within the non-diabetic range have been shown to be associated with an increased risk of cardiovascular disease and mortality, even after adjusting for other risk factors, including blood pressure, total cholesterol, high-density lipoprotein cholesterol (HDL-C), BMI, smoking status and physical activity.¹⁷ Therefore, the results of our study suggest that regularly breaking up prolonged sitting may have the potential to lower the risk of CVD in adults without diagnosed diabetes.

In the current study, five-hour interstitial glucose iAUC was 55.5% lower after sitting interrupted by light-intensity walking compared with after uninterrupted sitting. This finding adds to the existing evidence on the acute beneficial effects of regularly breaking up prolonged sitting with short bouts of light-intensity walking on blood glucose regulation.^{1,2,3,5} However, our effect size is considerably larger compared with previous studies, which reported reductions in postprandial glycemia ranging from 9.0%⁵ to 28.0%.³ One possible reason for this could be the different methods used to measure glucose concentration. Alternatively, it could be due to sample differences or the different methods used to calculate AUC.

Contrary to the weight of the existing evidence, Hansen et al. (2016)¹⁸ recently reported no difference in postprandial plasma glucose iAUC between 2.5 hours of uninterrupted sitting and sitting interrupted by two minutes of light-intensity walking every 20 minutes. However, the participants in this study were young and recreationally active, suggesting that light-intensity walking may not have been a sufficient stimulus. Furthermore, postprandial insulinemia was not measured, which may have been lower after the walking condition, despite no change in postprandial glycemia, due to an increase in insulin-independent (i.e. contraction-stimulated) glucose transporter 4 (GLUT4) translocation.¹⁹

Five-hour interstitial glucose iAUC was also 29.6% lower after sitting interrupted by standing compared with after uninterrupted sitting. However, the between-subject variability was large, with reductions in postprandial glycemia primarily limited to overweight men. Regularly breaking up prolonged sitting with short bouts of standing has previously been shown to lower postprandial glycemia in overweight, postmenopausal women with impaired glucose regulation.³ However, the current study is the first to show an acute beneficial effect in adults without metabolic impairment. Two previous studies in adults without metabolic impairment reported no effect of standing.^{1,5} However, the participants were younger and leaner than those in the current study and thus may have been more insulin sensitive.²⁰ These findings suggest that standing still for two minutes may only be a sufficient stimulus for insulin resistant individuals, with insulin sensitive individuals requiring longer standing bouts¹⁰ or additional movement during the standing bouts.⁸ In support of this, Skov-Jensen et al. (2007)²¹ found that 30 minutes of cycling (~40% VO_2 peak) elicited a greater increase in glucose uptake in adults with impaired glucose tolerance (IGT) compared with healthy controls, with the two groups matched on age and lean body mass.

Light-intensity walking was more effective than standing at lowering postprandial interstitial glucose concentration in the current study. This finding is in contrast to a previous

study, which reported similar reductions in postprandial glycemia following both sitting interrupted by standing and sitting interrupted by light-intensity walking.³ However, the participants were more insulin resistant than those in the current study, suggesting that the intensity of the activity breaks may be more important for insulin sensitive individuals.

Despite differences in glucose iAUC, there were no differences in glucose total AUC between the three trial conditions in the current study. Glucose total AUC has been shown to be strongly correlated with fasting plasma glucose concentration ($r = 0.90$), a marker of hepatic insulin sensitivity, whereas iAUC has been shown to be strongly correlated with postprandial glycemic rise ($r = -0.93$), a marker of skeletal muscle insulin sensitivity.²² Therefore, the absence of a condition effect for glucose total AUC suggests that the acute beneficial effects of regularly breaking up prolonged sitting may be localised to skeletal muscle, a finding that is consistent with a previous experimental study.²³

Both interventions for breaking up prolonged sitting in the workplace were found to be acceptable by the majority of participants in the current study. However, most participants preferred standing to light-intensity walking, primarily because it was less disruptive to their work. Future studies should examine the effects of breaking up seated office work less frequently, but for a longer period of time, as this is likely to be more feasible and sustainable in real life.

Strengths and limitations

The main strength of the current study is that we used a CGMS to record average interstitial glucose concentration every five minutes from the start of trial condition one until the end of trial condition three. This resulted in 61 glucose observations during each of the five-hour trial conditions, whereas previous studies only measured blood glucose concentration every 10 minutes,¹⁸ every 30 minutes^{3,5} or every hour.^{1,2} Therefore, our values

for glucose AUC are likely to be more accurate because shorter-term fluctuations in glucose concentration were taken into account. Furthermore, a larger number of data points allowed us to examine the postprandial and postabsorptive phases separately, as well as looking at the five-hour monitoring period as a whole. The effects of the two interventions may have been different during the postabsorptive phase, which is characterised by increased counter-regulatory hormone activity and hepatic glucose production.²⁴ However, we observed a similar pattern of results throughout.

Akintola et al. (2015)²⁵ showed good agreement between the iPro2 CGMS and simultaneous venous blood sampling for measuring glucose concentration in 34 normoglycemic adults (mean difference [95% limits of agreement], 0.10 mmol/L [-2.21 to 2.41 mmol/L]). However, continuous glucose monitoring (CGM) was associated with more random measurement error compared with venous blood sampling, suggesting that CGM studies may require larger sample sizes. Our sample size calculation was based on a study that used venous blood sampling. Therefore, the current study was underpowered because the between-subject variability within each trial condition was a lot larger than expected. As a result, the possibility of a Type 1 error cannot be ruled out, particularly for regular standing breaks.

CGMs measure glucose concentration in the interstitial fluid rather than in the blood. A time lag of four to 10 minutes has been observed between blood glucose and interstitial glucose.²⁶ However, this should not have affected the results of the current study because we were interested in the total glucose response to the test drinks rather than the time to peak glucose concentration. Wallace et al. (2006)²⁷ reported no difference in the total glucose response to a standard oral glucose load between the MiniMed CGMS and venous blood sampling.

Another strength of the current study is that it was conducted in the participants' workplaces rather than in a laboratory, increasing the ecological validity of its findings. This also enabled us to examine the acceptability, as well as the impact on perceived productivity, of two interventions that break up prolonged sitting in the workplace.

The current study also has a few limitations. Firstly, the participants were not blinded to trial condition order and therefore may have behaved differently on the day before each of the trial conditions. However, there were no differences in the total amount of time spent in MVPA and participants consumed a standardised evening meal at 7pm. Secondly, in order to prevent the insertion of more than one glucose sensor per participant, we used a 24-hour wash-out period between each trial condition rather than a minimum of four to seven days as used in previous studies.^{1-3,5,18} One hour of moderate-intensity cycling has been shown to enhance insulin sensitivity for up to 48 hours.²⁸ However, there were no differences in average interstitial glucose concentration on the day after each of the trial conditions (5.5 ± 0.3 , 5.5 ± 0.5 and 5.4 ± 0.4 mmol/L after sitting, standing and walking, respectively) and we adjusted for first-order carryover effects in the models. Thirdly, participants walked at a self-perceived light intensity around their workplace rather than at a fixed speed on a treadmill. This increased the ecological validity of the study, but may have contributed to the increased between-subject variability. That being said, postprandial glycemia was not influenced by walking pace in the study by Dunstan et al. (2012).² Finally, we did not measure postprandial insulinemia and therefore cannot infer whether less insulin was required to maintain glucose homeostasis in the two interrupted sitting trial conditions.

In conclusion, breaking up prolonged sitting every 20 minutes with two minutes of light-intensity walking lowers postprandial glycemia in middle-aged adults without metabolic impairment. Regular standing breaks may also be beneficial, particularly amongst adults who

are overweight or obese. Future studies should examine whether similar or greater effects are observed in adults with Type 2 diabetes.

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